Biodegradable implants for the biphasic pulsatile delivery of antigens

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Chapter 1

General introduction & Outline of this thesis

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GENERAL INTRODUCTION

Annually, vaccination saves at least 2-3 million lives worldwide [1,2] and is considered to be the most successful medical intervention in the prevention of infectious diseases [2]. Despite a great increase in vaccination coverage over the last decades [3], still an estimated 19.4 million infants (of which the vast majority in low- and middle-income countries) remained under-vaccinated in 2018, leaving them vulnerable to vaccine-preventable diseases [4]. A major factor contributing to this under-vaccination are the currently applied vaccination schedules.

Currently, most vaccines recommended by the World Health Organization require the administration of multiple doses at specific time intervals [5,6] in order to optimally protect the vaccinee against the pathogen due to, amongst other immune responses, the induction of increased and long-lasting serum antibody titers (schematically shown in Fig. 1 [7]). As a result, such a vaccination schedule, also known as the prime-boost regime [8], requires multiple visits to and/or by vaccination services. For several reasons, as described below, such a regime may not be followed successfully, which can finally lead to under-vaccination, especially in low- and middle-income countries.

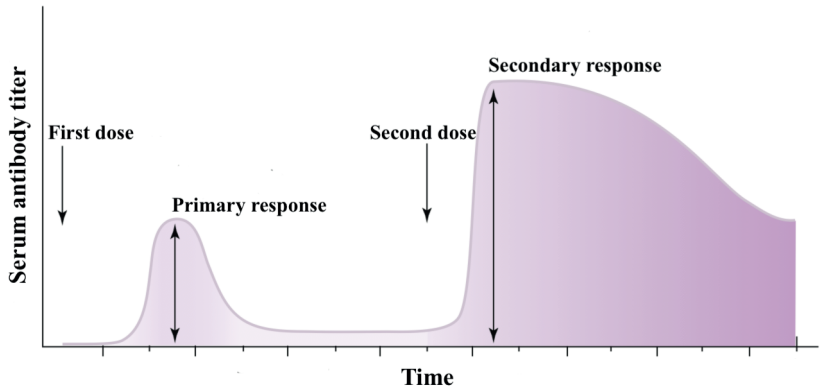

Fig. 1. The induction of increased and long-lasting serum antibody titers after two doses of the vaccine at specific time intervals [7].

In a systematic review, it has been shown that of the under-vaccinated children in low- and middle-income countries, 45% of the cases could be ascribed to logistical and/or financial problems. The four of these problems most frequently reported were: poor access and distance to vaccination services (23%), missed opportunity to vaccinate (21%), limited availability and knowledge of health workers (11%), and (direct and indirect) costs (11%) [9]. To decrease under-vaccination, these logistical and/or financial problems could be reduced by developing an injectable implant that mimics the currently applied prime-boost regime and optimally protects the vaccinee after receiving a single shot, also known as a single-injection vaccine.

The concept of a single-injection vaccine dates back to the 1970s and was

based on the sustained release (up to 100 days) of antigens that were monolithically incorporated into a non-biodegradable polymeric matrix [10,11]. To date, the controlled release of antigens from polymeric matrices is still considered to be the most promising approach for the development of a single-injection vaccine [12], however, research shifted toward the use of biodegradable polymers, as devices based on these polymers do not require surgical removal after release. Various biodegradable polymers could potentially be used for the development of a single-injection vaccine, with poly(DL-lactic-*co*-glycolic acid) (PLGA) being the most widely investigated [12,13].

PLGA is a biodegradable and biocompatible copolymer that consists of lactic and glycolic acid monomers. The polymer degrades via hydrolysis and has been used in many drug products that have been approved by the Food and Drug Administration [14]. A great advantage of PLGA is that its physicochemical characteristics (e.g. degradation rate and glass transition temperature) can easily be tailored by changing the lactic:glycolic acid ratio of the polymer [14]. Additionally, the physicochemical characteristics of PLGA can be tailored by changing the end group of the polymer and/or by using different molecular weights of the polymer [14]. Moreover, it has been shown that the release behavior of the incorporated active substance can be tailored by changing the physicochemical properties of the polymer [14,15].

A majority of the work on the development of PLGA-based single-injection vaccines is based on systems that exhibit continuous antigen release [12,13]. Although several of these continuous release single-injection vaccines showed positive results in animals, such a continuous release profile does not mimic the currently applied prime-boost regime and presents several potential disadvantages. First, it has been suggested that a continuous release of antigen may induce immune tolerance [13,16–18]. Second, a single-injection vaccine with continuous release kinetics may not be ideal from a regulatory approval standpoint as these kinetics do not mimic the currently approved prime-boost regimes [13]. To overcome these issues and to mimic the currently applied prime-boost regime, an implant with a pulsatile release could be developed.

Such a pulsatile release is characterized by discrete pulses of antigen release, each pulse representing a bolus immunization. The initial dose of the antigen, also known as the primer dose, could either be incorporated into the implant or applied onto the outer surface of the implant. To achieve the release of the booster dose, the system should be based on a delayed antigen release [12]. Ideally, the delay in release (also known as the lag time) of the system should easily be tailored, as different antigens have different vaccination schedules [5,6]. Successful development of a system that mimics the currently applied prime-boost regime and optimally protects the vaccinee after receiving a single shot could decrease under-vaccination and could save many lives annually, especially in low- and middle-income countries.

OUTLINE OF THIS THESIS

The overall aim of the research described in this thesis was to achieve a biphasic pulsatile release of antigens from biodegradable implants. Such a biphasic pulsatile release is characterized by the release of a part of the antigen immediately after administration, while the remaining part is released after a certain, preferably adjustable, lag time, thereby mimicking the currently applied prime-boost regime. To achieve this aim, prototypes based on two different concepts were investigated in this thesis.

The first prototype, evaluated in **chapter 2**, was based on the direct compaction of a physical mixture containing the antigen and either PLGA with a lactic:glycolic acid ratio of 50:50 (PLGA 50:50) or poly(DL-lactic acid) (PLA). In this chapter, the mechanism behind the biphasic pulsatile release of theophylline, as a model drug, from physically mixed compacts was investigated. PLGA 50:50 was compared with PLA to investigate whether the lag time prior to the delayed release could be tailored. In addition, instead of theophylline, blue dextran was incorporated to investigate whether the prototype may be suitable for the biphasic pulsatile delivery of bacterial polysaccharide-based antigens. Moreover, to investigate the effect of the molecular weight of the incorporated polysaccharide on the release behavior, blue dextran with either a molecular weight of 70 or 2000 kDa was incorporated. For the biphasic pulsatile delivery of protein-based antigens, this prototype was unfortunately unsuitable.

Therefore, the second prototype, a core-shell implant consisting of a nonporous PLGA shell around a solid-state core containing the antigen, was investigated for protein-based antigens. This concept was evaluated in **chapter 3**, where ovalbumin (OVA) as a model protein for protein-based antigens was incorporated into a solid-state core and encapsulated with PLGA. The work described in this chapter focused on the most challenging part of the biphasic pulsatile release, i.e. obtaining the delayed release of protein-based antigens after a certain, preferably adjustable, lag time. Relatively large (approximately 9 x 5 mm) core-shell compacts were used as a proof of concept. To tailor the lag time, three types of PLGA, differing in their lactic:glycolic acid ratio, were used. For this study, the OVA-containing core-shell compacts were evaluated *in vitro* and in BALB/c mice.

The core-shell concept may be interesting for the incorporation of protein-based antigens of various infectious diseases, including respiratory syncytial virus (RSV). To date, no vaccine against RSV is available, however, the viral envelope glycoproteins (in particular the fusion (F) protein) of the virus have shown great potential as protein-based antigens. **Chapter 4** gives a literature overview of RSV vaccine candidates based on the viral envelope glycoproteins intended for pregnant women and the elderly.

The main aim of the work described in **chapter 5** was to develop a stable freeze-dried powder containing the pre-fusion (pre-F) conformation of the RSV F protein in order to increase the flexibility regarding the route of administration of the antigen. In addition, the developed pre-F-containing powder was incorporated into a core-shell

compact as described in chapter 3 to investigate whether this system was suitable to obtain a delayed release of pre-F *in vitro* and in BALB/c mice. Finally, miniaturization of the core-shell compact was investigated *in vitro* by using injectable PLGA micro-tubes that were filled with aminophylline as a model drug.

The work described in **chapter 6** focused on obtaining an *in vitro* biphasic pulsatile release of protein-based antigens from the injectable PLGA micro-tubes described in chapter 5. To this end, the micro-tubes were filled with a powder containing OVA (booster). After filling, the micro-tubes were closed and the outer surface of the micro-tubes were coated with a fast dissolving thin layer containing OVA (primer).

Finally, **chapter 7** presents a general discussion of the obtained results. In addition, this chapter includes directions for future research.

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